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A redescription of *Schistura jarutanini* (Cypriniformes, Nemacheilidae) from the Mae Klong River Basin in western Thailand

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Abstract

Although originally described as a cave-inhabiting species, *Schistura jarutanini* is an epigean species rarely found in caves. Recent collections in the Mae Klong and Ataran River basins provide a more complete description of the species, type locality, relationships, and distribution. Among species for which molecular sequence data are available, *S. jarutanini* is mostly closely related to *S. mahnerti* with which it is sympatric. The two species are easily distinguished by color pattern and number of branched anal-fin rays. Headwater stream capture, or possibly underground dispersal, explains variation across lineages in *S. jarutanini* better than does dispersal within drainages.

Key Words

Phylogenetics, *Schistura mahnerti*, Southeast Asia

Introduction

Schistura jarutanini Kottelat, 1990 (Fig. 1) was described as a cave-inhabiting nemacheiline collected from an underground stream thought to be in the Khwae Yai drainage system in the Mae Klong River basin in western Thailand (Kottelat 1990). The type locality was given as Tham Ba Dan, but this is a general Thai phrase meaning subterranean cave. As noted by Jarutanin (1987), for whom the specimens were collected, the description of the species was based on specimens collected from Tham Sao Hin (14.7549°N, 98.9090°E) in Srisawat District, Kanchanaburi Province, Thailand. The stream from this cave joins the Khlong Ngu, a tributary of the Khwae Yai, one of the two major tributaries of the Mae Klong River. Tham Sao Hin is a large cave with fast-flowing water (Trajano et al. 2002) and has the tallest natural column in the world, at 61.5 m, the result of a joined stalactite and stalagmite (Guinness World Records Limited, 2025, London, England).

Recent collections of *S. jarutanini* allow for a more complete description of the species, an estimate of the phylogenetic relationships, and recognition that the species occurs primarily in surface streams in the Mae Klong and Ataran River basins and is not principally a cave-dwelling species.

Methods

Fishes were collected with nets, anesthetized, fixed in 10% formalin, and stored in 70% ethanol. Photographs of live and preserved specimens were taken with Canon EOS 7D and R cameras. All images of live specimens were taken

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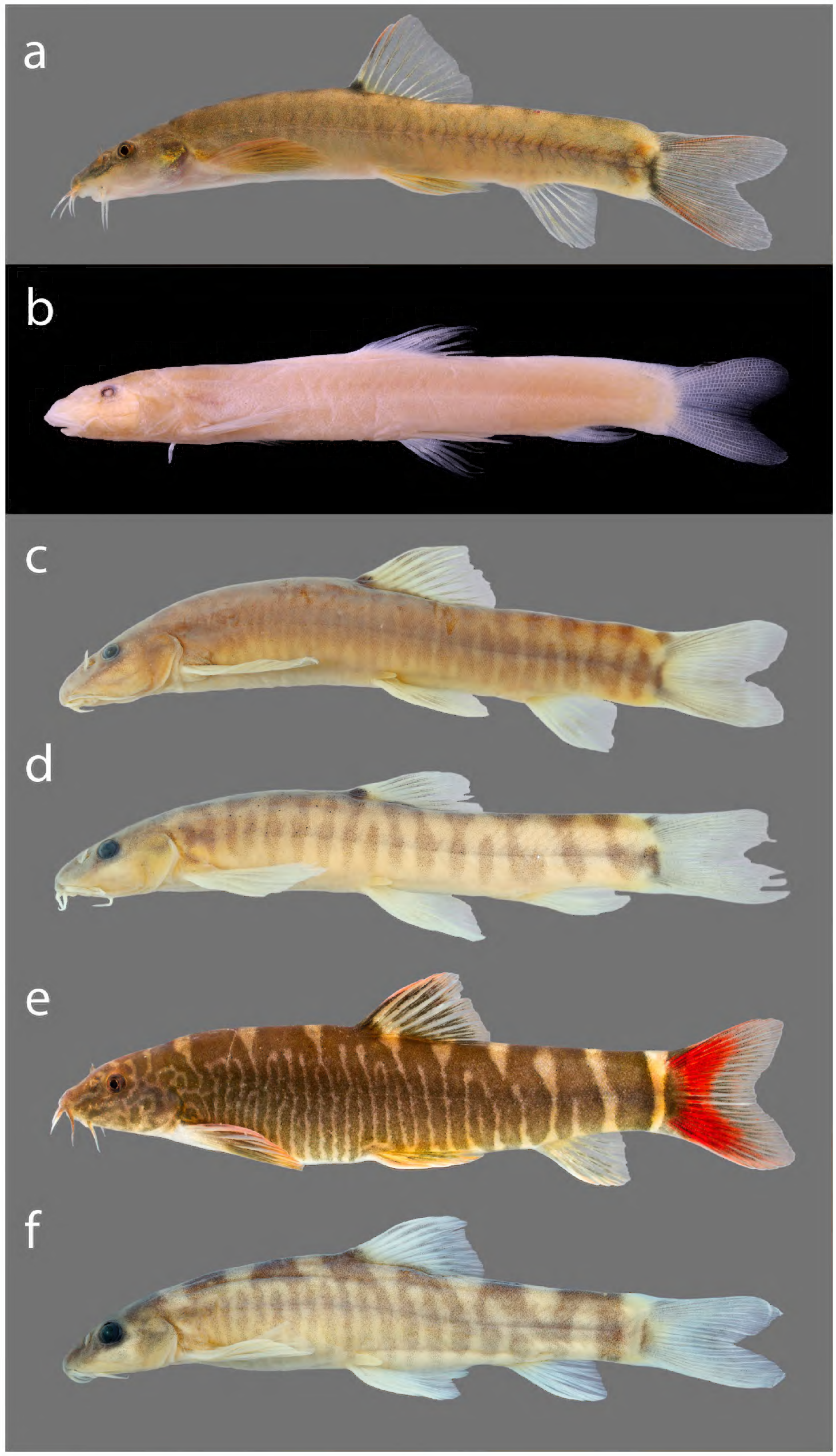

Figure 1. *Schistura jarutanini*: **a.** Live, UF 192380, 52.5 mm SL, Song Thai River, Kanchanaburi Province, Thailand; **b.** Paratype, ZMH 769, 68 mm SL, photo by Thilo Weddehage; **c.** Live, Thong Pha Phum, Kanchanaburi Province, Thailand, photo by Vatthanachai Phanklam, specimen not preserved; **d.** Preserved, UF 247882, 40.7 mm SL, Khaio River, Tak Province, Thailand. *Schistura mahnerti*: **e.** Live, UF 188061, 76 mm SL, tributary of Lake Vajiralongkorn, Kanchanaburi Province, Thailand; **f.** Preserved, UF 243489, 47.1 mm SL, Thong Pha Phum Creek, Kanchanaburi Province, Thailand.

soon after capture. Images were edited using Adobe Photoshop CC 2024 (Adobe, San Jose, CA). X-rays and computed tomography (CT) scans were generated using a Phoenix v|tome|x M scanner (GE Measurement & Control, Boston, MA) at the University of Florida's Nanoscale Research Facility. CT projections were processed using datos|x software v. 2.3 (Baker Hughes, Jacksonville, FL), and anatomy was segmented and visualized using VG StudioMax v. 2024.2.1 (Volume Graphics, Heidelberg, Germany). CT scans can be downloaded from MorphoSource (https://www.morphosource.org, *Schistura jarutanini*, UF 245434, Media ID 000568285 and 000568281; *S. mahnerti*, UF 246996, Media ID 000568249 and 000568245). Photos are by authors unless otherwise noted.

Molecular

Šlechtová et al. (2024) produced a phylogeny for the family Nemacheilidae from 471 samples of 279 species (some undescribed) from 37 genera, providing a large dataset for estimates of evolutionary relationships and taxonomic revisions. To the cytochrome *b* (cytb) and recombination-activating gene 1 (RAG1) sequences for 241 specimens from across the phylogeny from Šlechtová et al. (2024), we added new sequences for cytb and RAG1 and another mitochondrial gene, cytochrome *c* oxidase subunit 1 (COI) for 224 specimens of 60 species from nine genera. This produced a combined dataset for 465 specimens to examine relationships of *S. jarutanini*. Discussion of the total phylogenetic results generated is beyond the scope of this paper and will be published elsewhere. Relative to our target species, *S. jarutanini*, for which we added new sequences for 12 specimens, our multi-locus phylogeny produced the same sister-species relationship as Šlechtová et al. (2024). The closest relative of *S. jarutanini* is *S. mahnerti* Kottelat, 1990.

Subsequent analyses focused on relations among populations of *S. jarutanini* and *S. mahnerti* (Table 1). *Schistura aurantiaca* Plongsesthee, Page & Beamish, 2011 and *S. desmotes* (Fowler, 1934) were selected as outgroups based on phylogenetic relationships recovered in Šlechtová et al. (2024), with *S. aurantiaca* representing their "Southern Clade" and *S. desmotes* their "Indochinese Clade." GenSeq designations follow Chakrabarty et al. (2013).

Fin clips were taken from freshly captured fishes and preserved in 95% ethanol for DNA extraction. DNA was extracted using the Omega Bio-Tek E.Z.N.A Tissue DNA kit (Omega Bio-Tek, Norcross, GA) following manufacturer's instructions. Polymerase chain reactions (PCR) were run in 25 µL reaction volumes with 12.5 µL GoTaq Master Mix (Promega, Madison, WI), 9.5 µL water, 0.5 µL of each primer at 10mM, and 2 µL of DNA template. Mitochondrial cytochrome *c* oxidase subunit 1 (COI) was amplified by PCR and sequenced using the following primers: FishF1 5'TCAACCAACCACAAAGACATTGGCAC'3 and FishR2-5'ACTTCAGGGTGACCGAAGAATCAGAA'3 (Ward et al. 2005), and LCO1490-5'GGTCAACAAATCATAAAGATATTGG'3 and HCO2198-5'TAAACTTCAGGGTGACCAAAAAATCA'3 (Folmer et al. 1994). Mitochondrial cytochrome *b* (cytb) was amplified and sequenced using: 5'GAAGAACCACCGTTGTTATTCAA'3 and 5'ACCTCCRATCTYCGGATTACA'3 (Šlechtová et al. 2006), and 5'ATGGCAAGCCTACGAAAAAC3' and 5'TCGGATTACAAGACCGATGCTT3' (Yang et al. 2010). The nuclear recombination-activating gene 1 (RAG1) was amplified and sequenced using: 5'AGCTGTAGTCAGTAYCACAARATG'3 and 5'TCCTGRAAGATYTTGTAGAA 3' (Šlechtová et al. 2007). PCR cycles were completed with a MJ Research PTC-100 Thermal Cycler (GMI, Ramsey, MN) following the procedures outlined in the primer sources. To confirm the presence of the desired amplified sequence, gel electrophoresis was run on 1.5% agarose gel with 6 µL GelRed and TBE buffer. The PCR product was purified and sequenced by Molecular Cloning Laboratories (MCLAB, San Francisco, CA).

Chromatograms were edited, assembled using De Novo Assemble and aligned using MAFFT E-INS-l in Geneious Prime v. 2024.0.7 (Biomatters Ltd, Auckland, New Zealand; http://www.geneious.com). Bayesian Information Criterion (BIC) as implemented in IQ-TREE (Nguyen et al. 2015) was used to determine the best partition scheme and model of nucleotide evolution (GTR+F+G4). Maximum likelihood (ML) analyses were performed in IQ-TREE using the ultrafast bootstrap under a RAxML model (Stamatakis 2014) for each locus and the concatenated dataset for all three loci (COI, cytb, and RAG1).

Morphological

Morphological data were taken on 70 and 30 specimens of *S. jarutanini* and *S. mahnerti*, respectively, (see Material Examined) from western Thailand. Measurements were taken to the nearest 0.1 mm using digital calipers following Hubbs et al. (2004) except for predorsal-, prepectoral-, and preanal-fin lengths, all taken from the tip of the snout to the origin of the fin; head depth, taken at the posterior margin of the orbit; body depth and width, taken at the dorsal-fin origin. Total ray counts are given for paired fins, and branched ray counts for unpaired fins. For the last ray of the dorsal and anal fins, sharing a pterygiophore with the last branched ray, the count is given as ½. In addition to counts for specimens examined by us, meristic data include counts for the specimens of *S. jarutanini* examined by Kottelat (1990).

Proportional data are expressed as percentages of standard length (SL) or head length (HL). Vertebral counts were made on four individuals from CT scans. Counts of abdominal and total vertebrae include those in the Weberian complex (n = 4 vertebrae). Counts of caudal vertebrae began at the vertebra with its hemal spine just posterior to or in contact with the anterior anal-fin pterygiophore and include the urostyle complex (n = 1 vertebra).

Morphological variations between *S. jarutanini* and *S. mahnerti* and among populations of *S. jarutanini* were

Table 1. Voucher specimen and tissue data for sequences included in the molecular phylogeny. All samples are from Thailand. More locality data are provided in material examined. All samples are from this study except A778, A779, and NC-031584 from Šlechtová, et al. (2024). Are all GenSeq 4 except ICH-05977 is GenSeq 3 (Chakrabarty et al. 2013).

Species	Voucher	Tissue ID	River	Drainage	GenBank COI	GenBank Cyt b	Rag1
S. aurantiaca	UF 176400	2009-0697	Huay Kayaeng	Khwae Noi	PV982132	PV983947	PV983949
S. desmotes	UF 183066	2012-0343	Ping River	Chao Phraya	PV982133	PV983948	PV983956
S. jarutanini	UF 192380	ICH-02681	Song Thai River	Khwae Yai	PV982116	PV983940	–
S. jarutanini	UF 192380	ICH-02683	Song Thai River	Khwae Yai	PV982115	PV983941	–
S. jarutanini	UF 192404	ICH-02741	Song Thai River	Khwae Yai	PV982118	PV983943	PV983954
S. jarutanini	UF 192519	ICH-02735	Talusa River	Khwae Yai	PV982119	PV983942	–
S. jarutanini	UF 192519	ICH-02736	Talusa River	Khwae Yai	PV982114	PV983944	–
S. jarutanini	UF 192519	ICH-02740	Talusa River	Khwae Yai	PV982117	PV983945	PV983953
S. jarutanini	UF 243446	ICH-03622	Kroeng Krawia	Khwae Noi	PV982128	PV983938	–
S. jarutanini	UF 245464	ICH-04598	Chong Katok Creek	Khwae Noi	PV982112	–	–
S. jarutanini	UF 245434	ICH-04554	Kasat River	Ataran	PV982113	PV983946	–
S. jarutanini	UF 247882	ICH-03337	Khiao River	Khwae Yai	PV982131	–	–
S. jarutanini	UF 247882	ICH-03338	Khiao River	Khwae Yai	PV982130	–	–
S. jarutanini	–	ICH-05977	Khlong Ngu	Khwae Yai	PV982129	PV983939	–
S. jarutanini	GenBank	–	unknown	Mae Klong	–	NC-031584	–
S. mahnerti	A778	–	Salween	Salween	–	PP280030	PP315863
S. mahnerti	A779	–	Salween	Salween	–	PP280031	PP315864
S. mahnerti	UF 191785	ICH-03044	Mae Nam Noi Creek	Khwae Noi	PV982123	PV983935	PV983955
S. mahnerti	UF 191983	ICH-03067	Mae Phlu Creek	Khwae Yai	PV982121	PV983936	PV983952
S. mahnerti	UF 192379	ICH-02692	Song Thai River	Khwae Yai	PV982122	PV983934	–
S. mahnerti	UF 245355	ICH-04481	Ro Khi River	Khwae Noi	PV982120	–	–
S. mahnerti	UF 245437	ICH-04547	Kasat River	Ataran	PV982127	–	–
S. mahnerti	UF 245437	ICH-04546	Kasat River	Ataran	PV982126	–	–
S. mahnerti	UF 243489	ICH-03633	Thong Pha Phum Creek	Khwae Noi	PV982124	PV983937	PV983951
S. mahnerti	UF 246996	ICH-02634	Khiao River	Khwae Yai	PV982125	PV983933	PV983950

assessed through standardized principal component analyses (PCA), which allowed for comparison independent of standard length (Humphries et al. 1981; Rohlf 1999). Principal component 2 (PCII) and principal component 3 (PCIII) were calculated using JMP® Pro v18.1.2 (SAS Institute Inc. 2024) and plotted using R v4.3.3 (R Core Team 2024) with packages *ggplot2* v3.5.0 (Wickham 2016) and *ggforce* v0.4.2 (Pedersen 2024). Log-transformed correlation matrices were generated from measurements of 13 traits from 99 specimens.

Results

Molecular

Phylogenetic analyses examining relationships within *S. jarutanini* included samples from eight localities in the Khwae Yai and Khwae Noi, major tributaries of the Mae Klong River which flows to the Gulf of Thailand, and from the Kasat River which joins the Ataran River and flows to the Andaman Sea. For the sister species, *S. mahnerti*, eight localities from the Ataran, Khwae Noi, Khwae Yai, and Salween River basins were represented (Table 1).

In the three-locus tree (Fig. 2), four major lineages were recognized for *S. jarutanini* samples. One was from the Khiao River which is far upstream in the Khwae Yai drainage, and a second included six specimens from the Khwae Yai, one from the Khwae Noi, and one from the Kasat River. The third lineage had one specimen from the Khwae Noi and one from Khlong Ngu, the stream emanating from the type locality in the Khwae Yai basin. The fourth lineage was from GenBank and included in the Šlechtová et al. (2024) phylogeny from an unknown locality (originally obtained by Misaka Miya, personal communication). The single-locus mitochondrial trees, which had low support values, and the Rag1 tree which included only two samples for *S. jarutanini* are not shown.

Morphological

Variations in body shape and color patterns of *S. jarutanini* and *S. mahnerti* are shown in Fig. 1 and relative proportional values are in Table 2. In the sheared principal component analysis of shape differences between *S. jarutanini* and *S. mahnerti*, PC1, which was heavily influenced by size and not plotted, accounted for 93.4% of observed variance. The second principal component accounted for 2.9% of variance and the third for 1.2%. Eye diameter (0.45) and interorbital width (-0.36) had the highest loadings on PC2. Caudal-peduncle length (-0.28), pectoral-fin length (0.16), and pelvic-fin length (0.15) had the highest loading on PC3. *Schistura jarutanini* separated from *S. mahnerti* on PC2 and slightly on PC3 (Fig. 3).

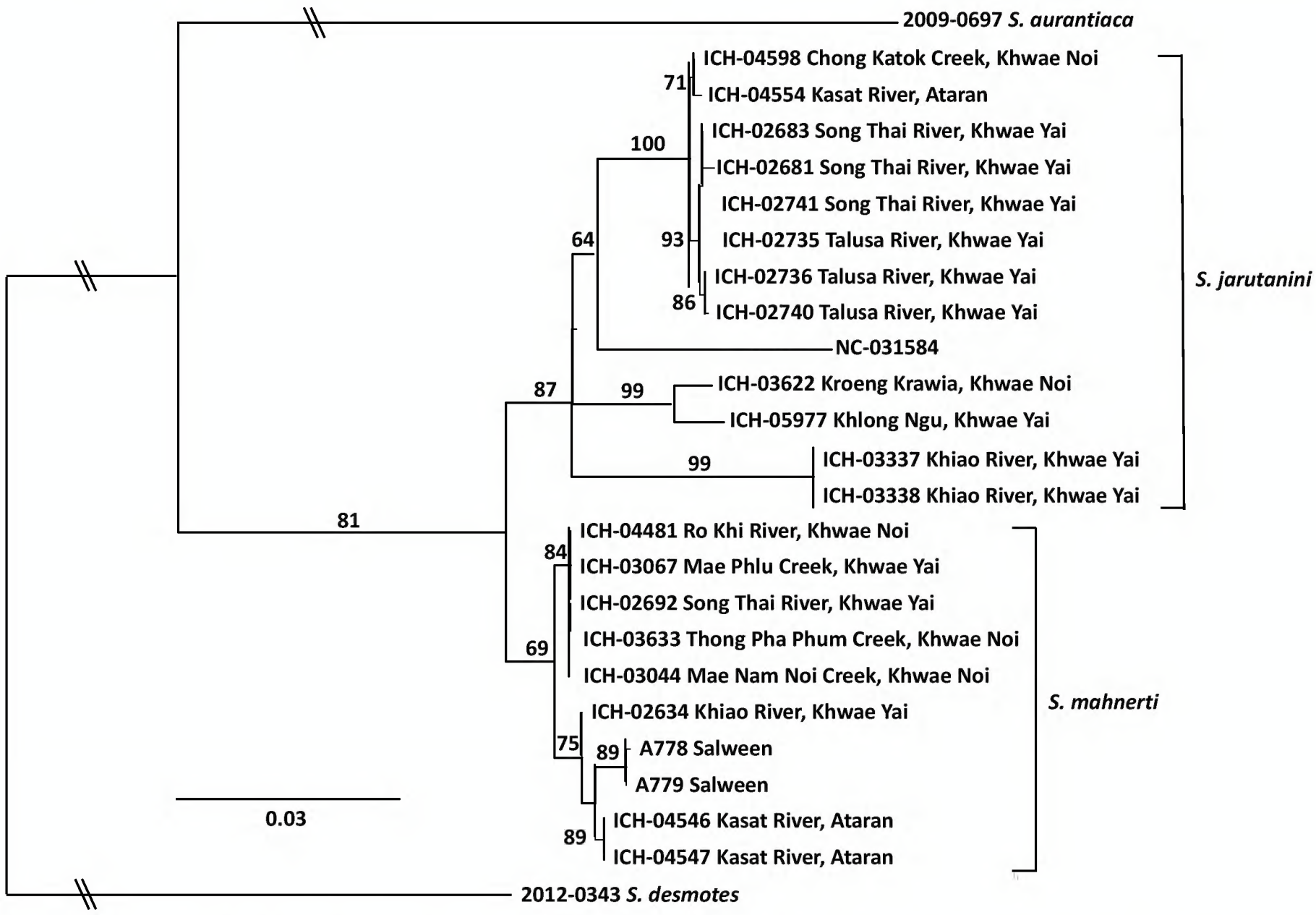

Figure 2. Maximum likelihood phylogenetic reconstruction for the concatenated three-loci dataset showing relationships among *S. jarutanini* and *S. mahnerti*.

Table 2. Proportional data for *Schistura jarutanini* and *S. mahnerti*. Numbers of specimens in parentheses.

	***S. jarutanini* (UF, N = 69)**			***S. jarutanini* (Kottelat, 1990, N = 17)**			***S. mahnerti* (UF, N = 30)**		
	range	**mean**	**SD**	**range**	**mean**	**SD**	**range**	**mean**	**SD**
Standard length (mm)	22.6–62.0	38.5	9.60	55.5–67.2	–	–	27.7–62.5	44.9	9.57
% SL									
Predorsal length	49.4–58.1	53.0	1.79	49.6–53.3	51.8	1.04	49.7–56.3	52.9	1.84
Prepelvic length	49.0–57.1	52.7	1.83	54.8–59.8	56.7	1.47	48.9–56.3	52.3	1.71
Preanal length	75.5–83.2	79.6	1.68	78.7–83.2	81.1	1.32	75.1–82.2	79.1	1.77
Body depth	13.1–18.9	15.8	1.28	13.4–16.9	15.7	0.94	14.2–20.8	17.5	1.61
Caudal-peduncle depth	9.9–13.9	11.5	0.80	10.0–12.4	11.4	0.75	9.9–15.7	12.9	1.41
Caudal-peduncle length*	11.8–18.9	14.9	1.75	9.7–13.7	11.9	1.27	11.0–17.9	15.1	1.53
Pelvic-fin length	19.4–24.7	21.9	1.17	19.0–20.2	19.6	0.39	18.8–23.3	20.9	1.16
Pectoral-fin length	22.0–28.9	25.1	1.33	22.8–24.3	25.3	0.51	21.6–26.2	23.7	1.13
Head length	22.6–28.2	24.7	1.11	23.4–25.3	24.3	0.57	22.6–26.5	24.5	0.90
Snout length	9.1–11.8	10.5	0.65				9.0–12.3	10.3	0.75
Eye diameter	3.2–6.1	4.8	0.73				4.1–6.0	5.0	0.47
Interorbital width	6.6–9.7	7.7	0.78				5.7–7.4	6.5	0.49
% HL									
Snout length	35.0–49.2	42.6	2.91				36.0–46.6	42.1	2.47
Eye diameter	13.5–24.3	19.6	2.52				16.0–24.6	20.3	1.87
Interorbital width	25.0–37.7	31.4	3.10				21.6–30.6	26.7	2.06

*Perhaps measured differently by Kottelat (1990).

Shape differences among populations of *S. jarutanini* were examined using principal component analysis by major drainage (Fig. 4a), i.e., Khwae Noi-red, Khwae Yai-green, and Kasat River-blue, and by major lineages (Fig. 4b) recognized in the phylogenetic analysis (Fig. 2), i.e., lineage 1 = Khwae Noi and Khlong Ngu, lineage 2 = Khwae Yai, Khwae Noi, and Kasat River, and lineage 3 = Khiao River upstream in the Khwae Yai drainage. PC1,

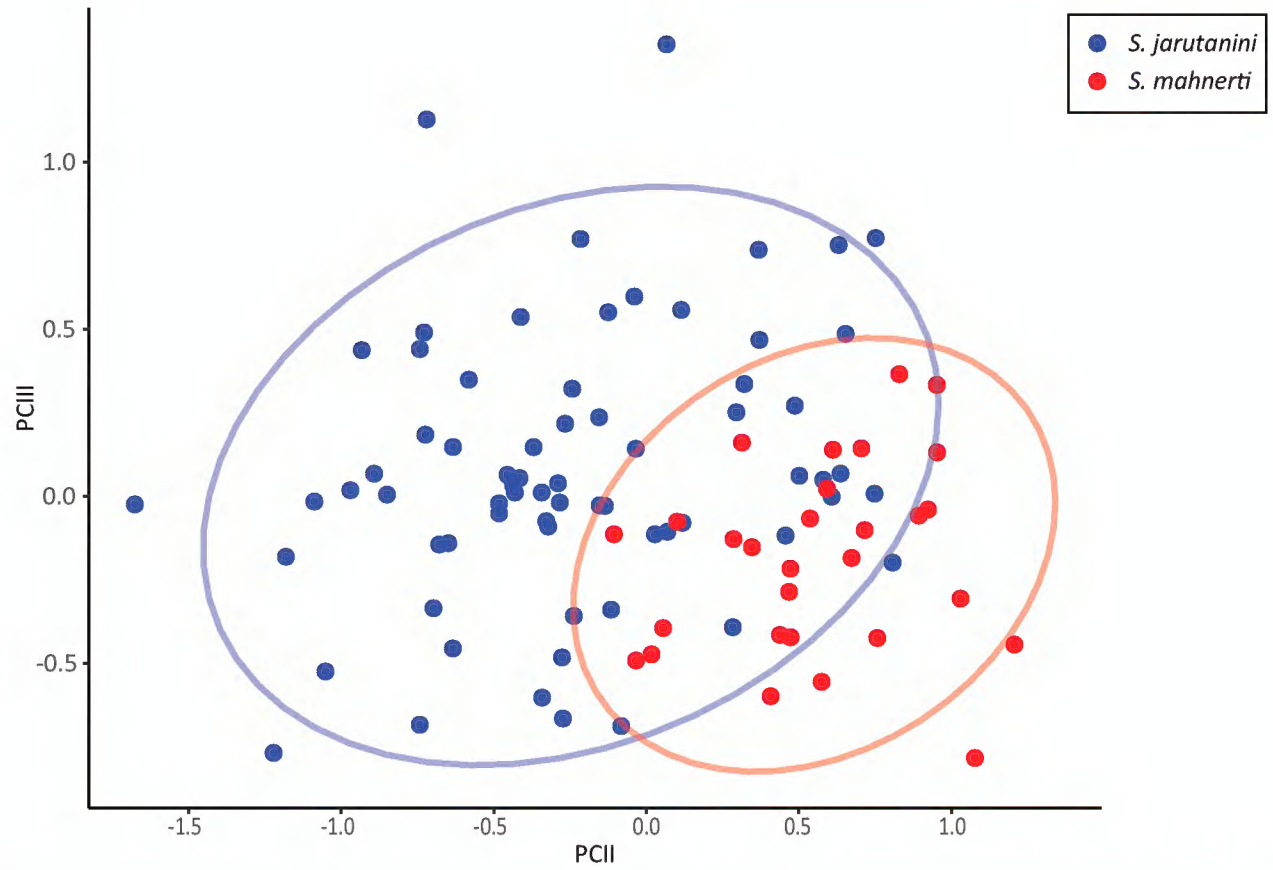

Figure 3. Principal component analysis for the log-transformed correlation matrix of morphological data for *S. jarutanini* and *S. mahnerti*.

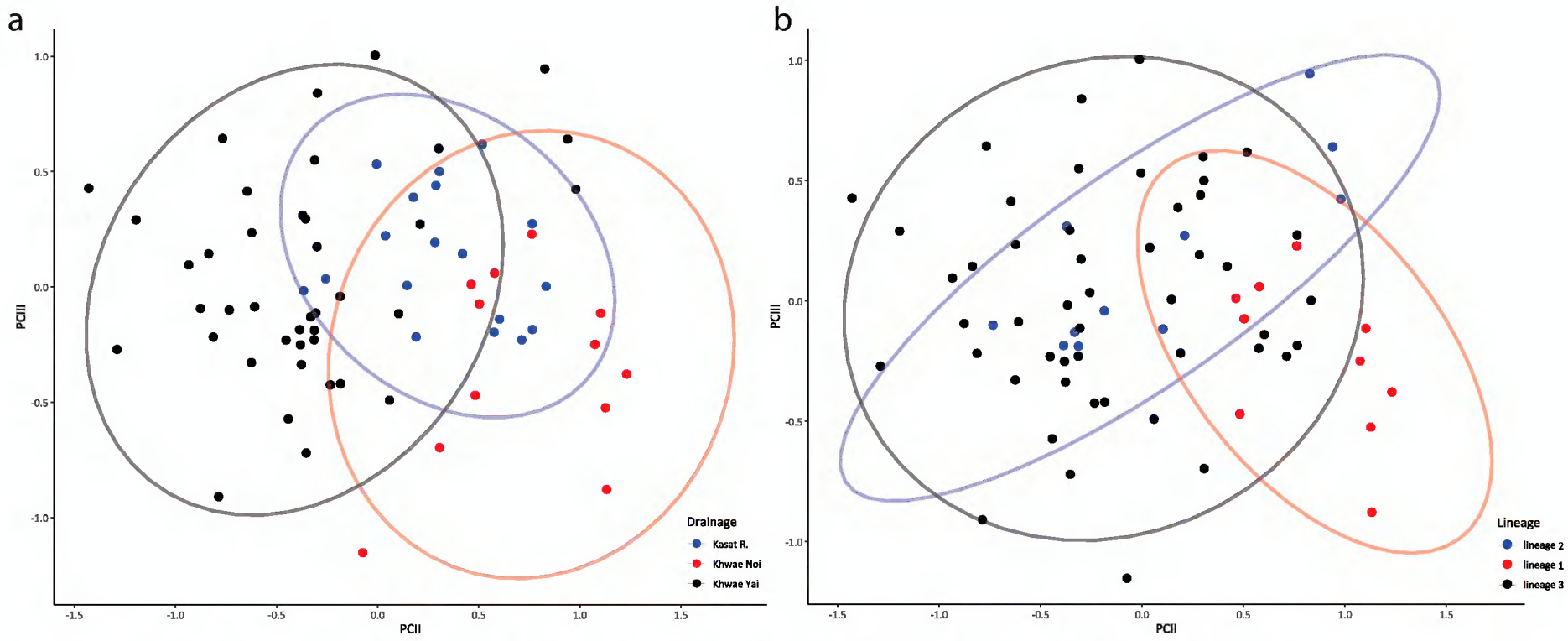

Figure 4. Principal component analysis for the log-transformed correlation matrix of morphological data for *S. jarutanini* by (**a**) major drainage and (**b**) major lineage.

which was heavily influenced by size and not plotted, accounted for 98.7% of observed variance. The second principal component accounted for 0.6% of variance and the third for 0.2%. Mandibular barbel length (0.231), maxillary barbel length (0.210), and eye diameter (0.173), had the highest loadings on PC2; mandibular barbel length (0.114), anal-fin length (0.080), and eye diameter (-0.072) had the highest loading on PC3.

The largest separation among drainages was between Khwae Noi and Khwae Yai on PC2 with the Kasat River overlapping the other two drainages. The largest separation of lineages was on PC2 between lineage 1 (Khwae Noi and Khlong Ngu) and the other two lineages, which failed to separate from one another on PC2 or PC3.

Schistura jarutanini and *S. mahnerti* consistently had 8½ branched dorsal-fin rays and 17 (9 upper, 8 lower) branched caudal-fin rays. *Schistura jarutanini* consistently had 5½ branched anal-fin rays, while *S. mahnerti* had 6½. Specimens of both species usually had 11 or 12 pectoral-fin rays and 8 pelvic-fin rays (Tables 3, 4). However, specimens of *S. jarutanini* from the Khwae Yai tended to have 12 pectoral-fin rays and 8 pelvic-fin rays, whereas those from the Khwae Noi more often had 11 pectoral-fin and 7 pelvic-fin rays, and those from the Kasat had 11 or 12 pectoral-fin rays and 8 pelvic-fin rays (Tables 3, 4). Specimens of *S. mahnerti* from the Khwae Yai tended to have 11 pectoral-fin rays and 8 pelvic-fin rays, whereas those from the Khwae Noi and Kasat River tended to have 12 pectoral-fin and 8 pelvic-fin rays.

Schistura jarutanini lacks red on the fins and a wide light-colored bar just before the base of the caudal fin, has a black bar mostly restricted to the lower half of the caudal-fin base and a black spot on the unbranched rays at the dorsal margin of the caudal fin. In contrast, the caudal fin of *S. mahnerti* is bright red and the bar at the end of the caudal peduncle is cream-colored (Fig. 1).

Table 3. Frequencies of pectoral-fin and pelvic-fin ray counts in *Schistura jarutanini* and *S. mahnerti* by drainage.

Species	Number of pectoral-fin rays							Number of pelvic-fin rays					
	10	**11**	**12**	**13**	**N**	**Mean**	**SD**	**7**	**8**	**9**	**N**	**Mean**	**SD**
S. jarutanini													
Khwae Yai	1	11	23	4	39	11.8	0.66	0	39	0	39	8	0.00
Khwae Noi	0	8	4	0	12	11.3	0.47	9	3	0	12	7.3	0.43
Kasat	0	9	9	0	18	11.5	0.50	0	18	0	18	8	0.00
Total	**1**	**28**	**36**	**4**	**69**	**11.6**	**0.62**	**9**	**60**	**0**	**69**	**7.9**	**0.34**
S. mahnerti													
Khwae Yai	1	8	2	0	11	11.1	0.51	0	11	0	11	8	0.00
Khwae Noi	0	3	11	0	14	11.8	0.43	5	8	1	14	7.7	0.61
Kasat	0	2	8	0	10	11.8	0.40	0	10	0	10	8	0.00
Total	**1**	**13**	**21**	**0**	**35**	**11.6**	**0.56**	**5**	**29**	**1**	**35**	**7.9**	**0.40**

Table 4. Frequencies of pectoral-fin and pelvic-fin ray counts in *Schistura jarutanini* by lineage (Figs 2, 7).

Lineage	Number of pectoral-fin rays							Number of pelvic-fin rays				
	10	**11**	**12**	**13**	**N**	**Mean**	**SD**	**7**	**8**	**N**	**Mean**	**SD**
Northern	0	7	2	1	10	11.4	0.66	0	10	10	8.0	0.00
Central (except Kasat)	1	4	23	3	31	11.9	0.59	0	31	31	8.0	0.00
Central (Kasat)	0	9	9	0	18	11.5	0.50	0	18	18	8.0	0.00
Southern	0	8	2	0	10	11.2	0.40	9	1	10	7.1	0.30
Total	**1**	**28**	**36**	**4**	**69**	**11.6**	**0.62**	**9**	**60**	**69**	**7.9**	**0.34**

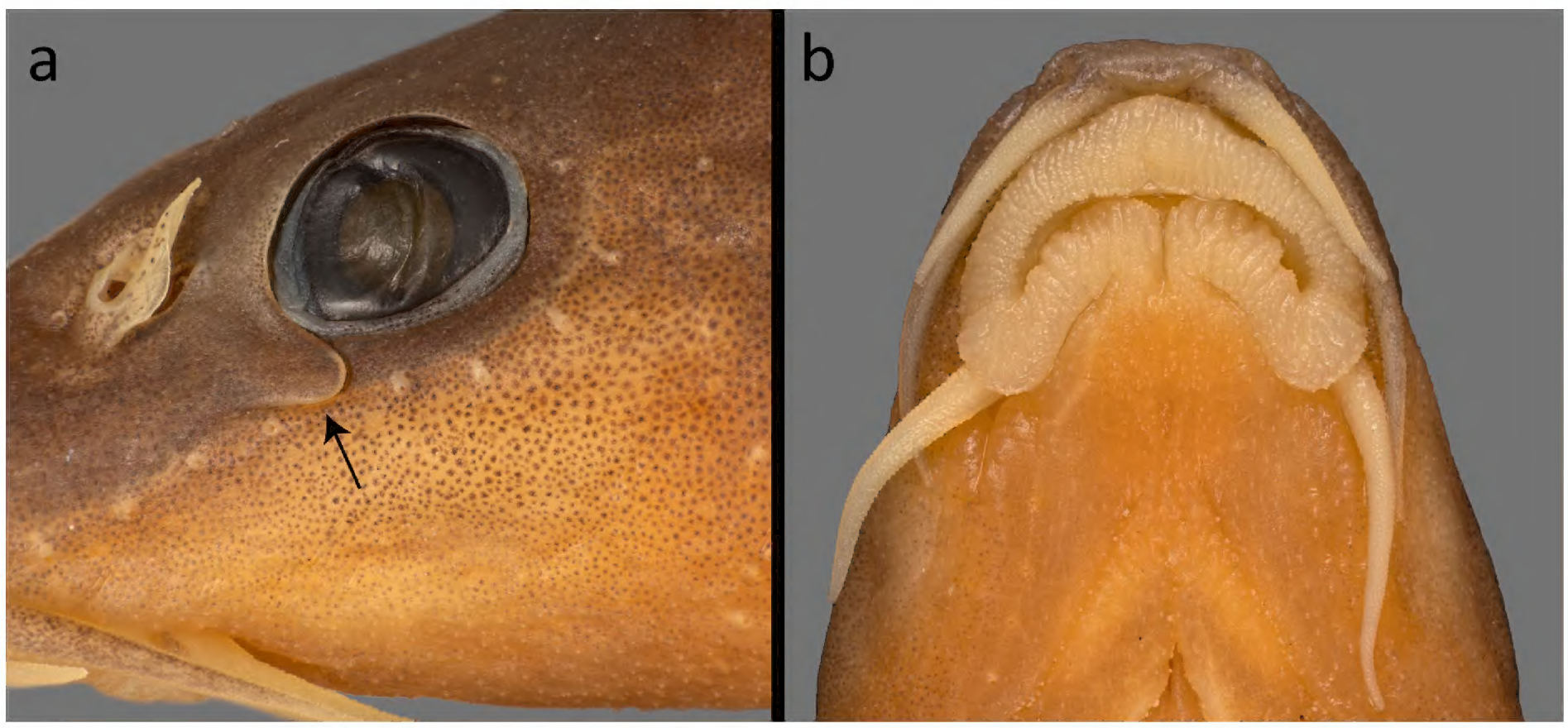

Figure 5. *Schistura jarutanini*, UF 245316, 48 mm SL, male, Kasat River, Kanchanaburi Province, Thailand, **a.** suborbital flap, **b.** mouth.

Schistura mahnerti may have a black bar on the caudal-fin base but lacks a discrete black spot on the unbranched rays above it.

Both species have a complete or nearly complete lateral line, an axillary pelvic-fin lobe, and furrowed lips with a small median incision in the upper lip and a median interruption in the lower lip (Fig. 5). The two halves of the air bladder capsule are connected medially by a manubrium (Fig. 6). Males of both species have a suborbital flap (Fig. 5), and females may have a suborbital slit. *Schistura jarutanini* has 22 abdominal + 13 caudal = 35 total vertebrae (UF 245434, N = 2); *S. mahnerti* has 20 + 13–14 = 33–34 vertebrae (UF 246996, N = 2). Other osteological differences between the two species have not been investigated in detail, but CT scans (N = 2 per species) (Fig. 6) show substantial differences including *S. jarutanini* having 5–6 (vs. 9–10 in *S. mahnerti*) dorsal and 4–5 (vs. 6) ventral procurrent rays, proximal portion of the anterior ribs straight (vs. curved), and anterior portion of the frontal bone V-shaped (vs. U-shaped).

Distribution

Schistura jarutanini is found in creeks and small to mid-size rivers over gravel and cobble in the Khwae Yai and Khwae Noi, the two major tributaries of the Mae Klong, which flows south to the Gulf of Thailand, and in the Kasat

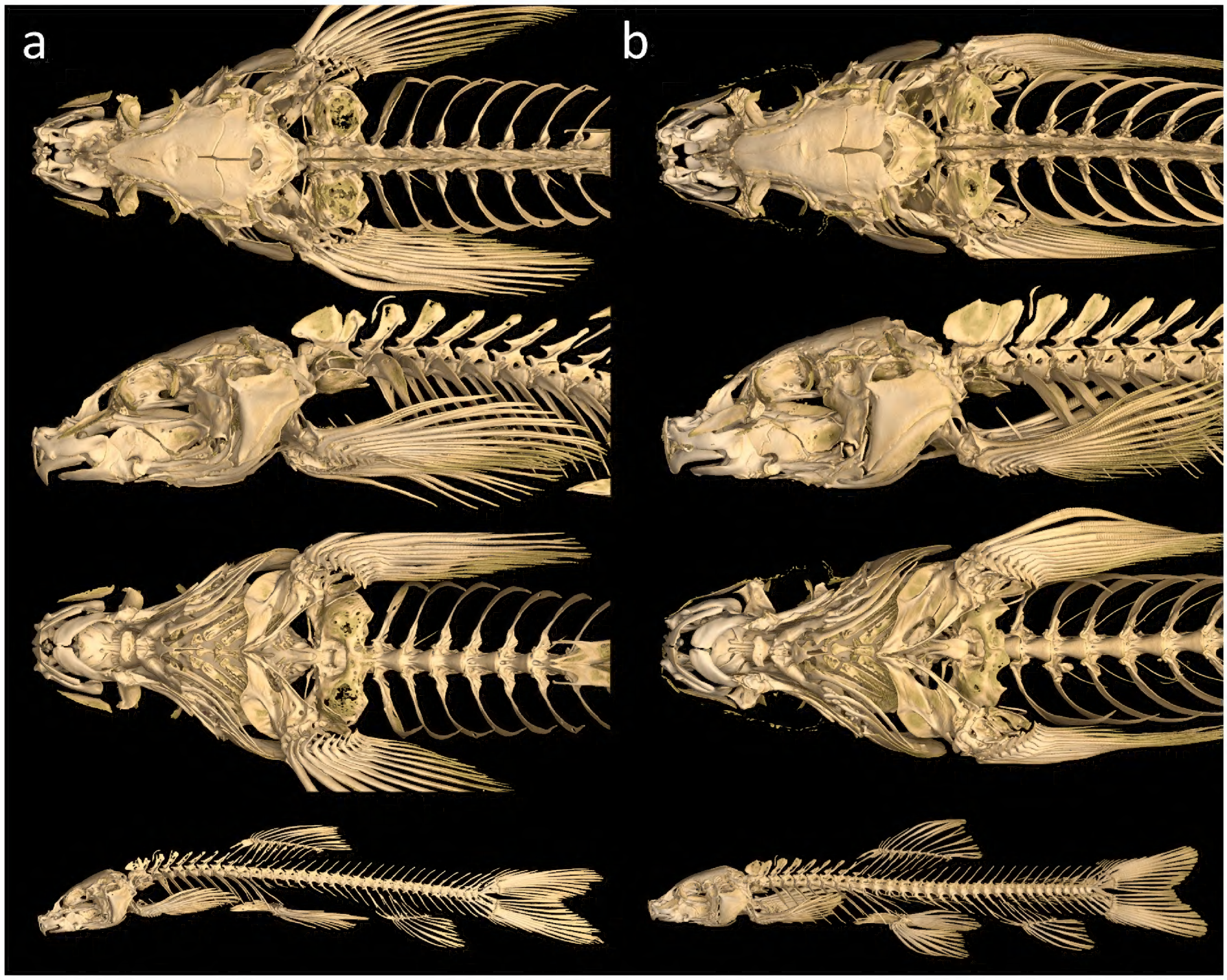

Figure 6. CT scans: **a.** *Schistura jarutanini*, UF 245434, 51.7 mm SL, Kasat River, Kanchanaburi Province, Thailand; **b.** *Schistura mahnerti*, UF 246996, 56.7 mm SL, Nam Khiao, Tak Province, Thailand.

River flowing westward to the Ataran River and the Adaman Sea (Fig. 7). Although all known specimens examined by us are from Thailand, the species is expected in the Ataran River basin in Myanmar.

Discussion

Although originally described as a cave-inhabiting species from a single locality and with eyes reduced or absent and a light color pattern, *S. jarutanini* is primarily an epigean species with normal eyes and dark pigmentation occurring over a moderately large area in the Mae Klong and Ataran River basins in Kanchanaburi and Tak Provinces, Thailand and based on institutional collections is rarely found in caves. The epigean nature of *S. jarutanini* was noticed previously by Trajano et al. (2002) who found only fully pigmented individuals at the type locality of *S. jarutanini*. Duboué and Borowsky (2012) studied rest-activity patterns in five individuals of *S. jarutanini* from the type locality but did not comment on pigmentation.

Populations of epigean species inhabiting caves often have reduced eyes and dark pigment, and some species appear to maintain populations in both environments. The most-studied is the Mexican Tetra, *Astyanax mexicanus* (De Filippi, 1853), with sighted and pigmented surface-dwelling forms and blind depigmented cave-dwelling forms (Jeffery 2009). Eye primordia are present in embryos of cave forms but atrophy without developing into functional eyes. The absence of dark pigmentation is due to the inability of undifferentiated melanoblasts to accumulate L-tyrosine, the precursor of melanin. Many examples of mostly epigean fishes with reduced eyes and pigmentation in cave environments are known, including another species in the Mae Klong basin, the silurid, *Pterocryptis buccata* Ng & Kottelat, 1998.

Although a suborbital flap usually occurs only in male nemacheilids, all 17 specimens examined by Kottelat (1990) had a suborbital flap, and the presence of the flap in both sexes was said to distinguish *S. jarutanini* from all other species of *Schistura*. Of the 70 specimens we examined, 26 (26.8–54.5 mm SL) had a flap, and 44 (22.6–62.0 mm SL) did not, with the latter being females or juvenile males. It may be that Kottelat (1990) examined only adult males (all were over 55.5 mm SL), or perhaps selection in the cave environment favored a flap in both sexes.

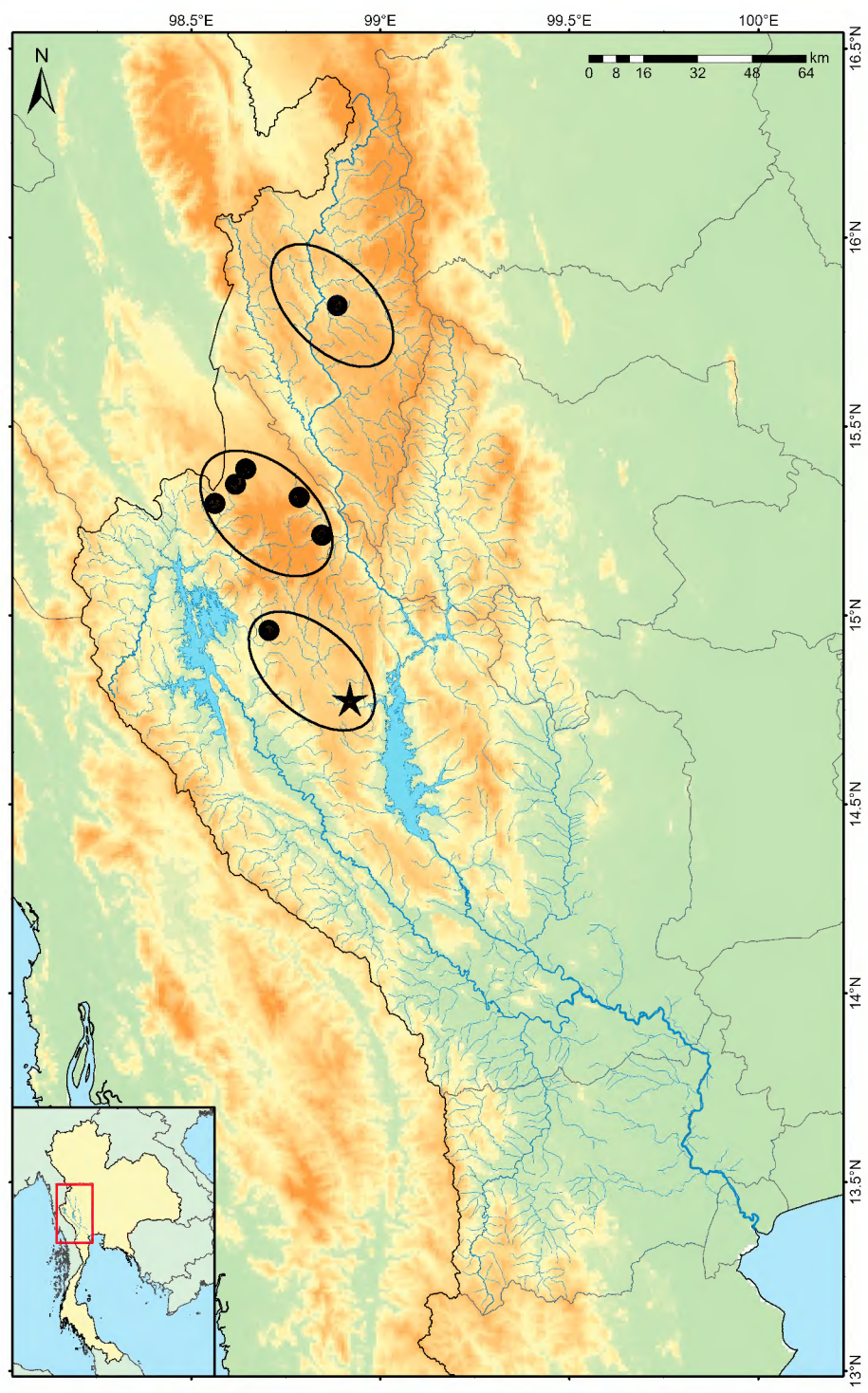

Figure 7. Mae Klong River basin in western Thailand with localities of *S. jarutanini* examined; star = type locality, encirclements correspond to lineages in Fig. 2.

Schistura jarutanini showed considerable genetic structure in the phylogenetic analysis (Fig. 2) with four principal lineages. Surprisingly, two of the lineages linked populations across drainages rather than only within a drainage. The "Southern" lineage (southern encirclement in Fig. 7) included specimens from Kroeng Krawia in the Khwae Noi drainage and Khlong Ngu, the stream emanating from the type locality in the Khwae Yai drainage. The "Central" lineage (central encirclement in Fig. 7) included specimens from three drainages, the Khwae Yai, Khwae Noi, and Kasat River. In contrast, the "Northern" lineage (northern encirclement in Fig. 7) included specimens only from Khiao River, far upstream in the Khwae Yai drainage and geographically distant from other lineages. The fourth lineage (NC-031584) was based on a sequence from GenBank with an unknown locality and no voucher or morphological data.

For the Southern, Central, and Northern lineages, the genetic result was mirrored by the variation in number of paired-fin rays, with each lineage having a unique signature (Table 4). The Northern lineage had modally 11 pectoral-fin rays and 8 pelvic-fin rays. The Southern lineage had modally 11 pectoral-fin rays and 7 pelvic-fin rays. The Central lineage (with specimens from Kasat River flowing to Adaman Sea excluded) had modally 12 pectoral-fin rays and 8 pelvic-fin rays. Kasat River specimens had 11–12 pectoral-fin rays, rendering it intermediate in paired-fin counts. Specimens from the Mae Klong basin can be assigned to one of the three genetic lineages by its pectoral- and pelvic-fin ray counts with a high degree of accuracy; 70% of Northern specimens have a pectoral- and pelvic-fin ray count of 11 and 8, 74% of Central specimens have a count of 12 and 8, and 70% of Southern specimens have a count of 11 and 7. Less distinctiveness was expressed among lineages in shape, as examined in the PCA, with only the Southern lineage showing some separation (Fig. 4b). This is perhaps not surprising with shape features such as head length, body depth, etc., likely less labile than meristic counts in fast-flowing rocky streams.

In comparing separation by drainage with that by lineage, specimens from the Khwae Yai had modally 12 pectoral-fin rays and always 8 pelvic-fin rays, those from the Khwae Noi had modally 11 pectoral-fin rays and modally 7 pelvic rays, and those from Kasat River had 11 or 12 pectoral-fin rays in equal numbers and always 8 pelvic-fin rays. Although specimens from the Kasat River could not be identified by drainage, counts allowed a plurality of specimens in the Khwae Yai (59%) and Khwae Noi (67%) to be assigned to one of the two drainages. Likewise, in the PCA the largest separation among drainages (Fig. 4a) was between Khwae Noi and Khwae Yai with the Kasat River overlapping the other two drainages.

Although variation within a species is expected to show affinity to river drainage because of easy dispersal within, but difficult dispersal across drainages, all datasets for *S. jarutanini* show the intermediacy of the Kasat River population of *S. jarutanini*. These results are unexpected given Khwae Noi and Khwae Yai are tributaries of the Mae Klong and drain to the Gulf of Thailand, whereas Kasat River drains to the Salween River and then to the Andaman Sea. However, the results can be explained by stream-capture dispersal between the Kasat and the Khae Noi, between the Kasat and the Khwae Yai, or between the Kasat and both the Khwae Noi and Khwae Yai. Headwaters of the three rivers interdigitate in the mountainous area in northern Kanchanaburi Province suggesting abundant opportunities for stream capture. Alternatively, given the species' occasional occupation of underwater (cave) stream environments, the dispersal may have occurred, at least in part, subterraneously. Headwater stream capture or underground dispersal explain intraspecific variation and distribution in *S. jarutanini* better than does dispersal within drainages alone. Combinations of underground dispersal and surface migration have been hypothesized to explain distributions of other fishes, e.g., the North American amblyopsid cavefishes (Niemiller and Poulson 2010). Ecological as well as more molecular and morphological data are required to fully understand the complex life-history and distribution of *S. jarutanini*.

Conclusion

Recent fieldwork indicates that *S. jarutanini* is primarily an epigean species, not a localized cave species as originally described. Phylogenetic analysis of combined mitochondrial and nuclear sequences for 465 specimens revealed the closest relative of *S. jarutanini* to be *S. mahnerti* from which it differs most trenchantly in color pattern and branched anal-fin rays. *Schistura jarutanini* occurs in a moderately large area of the upper Khwae Noi and central and upper Khwae Yai drainages in the Mae Klong basin flowing to the Gulf of Thailand and in upper reaches of the Kasat River flowing to the Andaman Sea. Headwater stream capture or underground dispersal explain intraspecific variation and distribution in *S. jarutanini* better than does dispersal within drainages alone.

Material examined

All specimens are from Thailand. Scientific name is followed by river basin, province, institutional catalog number, number of specimens examined, range in standard length in mm, water body, district, and geocoordinates ("est." if estimated herein). Abbreviations for institutional collections are: NIFI–National Inland Fisheries Institute, Bangkok, Thailand; UF–University of Florida, Florida Museum of Natural History, Gainesville, FL, USA; ZMH–Zoologisches Institut und Zoologisches Museum der Universität Hamburg.

***Schistura jarutanini*:** *Mae Klong basin*: Kanchanaburi Province: NIFI 03039, paratype, 53 mm SL, Tham Sao Hin cave at Tham Ba Dan, Srisawat District, 14.76688°N, 98.90819°E (est.); UF 192404, 9, 28.1–62.0 mm SL, Song Thai River behind Maharaja field station in Thung Yai Wildlife Sanctuary, Thong Pha Phum District, 15.21071°N, 98.85458°E; UF 192519, 10, 28.9–49.9 mm SL, Talusa Creek at Sesavo Station, Sangkhla Buri District, 15.32878°N, 98.78156°E; UF 245464, 2, 42.6–45.5 mm SL, Chong Katok Creek in Thung Yai Naresuan Wildlife Sanctuary, Sangkhla Buri District, 15.28091°N, 98.56477°E; UF 192380, 10, 22.6–52.5 mm SL, Song Thai River behind Maharaja field station in Thung Yai Wildlife Sanctuary, Thong Pha Phum District, 15.21071°N, 98.85458°E; UF 243446, 10, 26.2–50.4 mm SL, tributary of Kroeng Krawia at Ban Thi Phu Ye, Thong Pha Phum District, 14.95265°N, 98.691°E. ZMH 7962, paratypes, 51.3–66.5 mm SL (photos only) same data as NIFI 03039. Tak Province: UF 247882, 10, 21.3–47.2 mm SL, Khaio, Umphang District, 15.81472°N, 98.87881°E. *Ataran basin*: Kanchanaburi Province: UF 245434, 10, 31.2–52.8 mm SL, Kasat River at Ban Thi Rai Pa, Sangkhla Buri District, 15.37552°N 98.58283°E; UF 245316, 8, 31.4–50.6 mm SL, Kasat River at field station 5.5 kilometers NE of Ban Thi Rai Pa, Sangkhla Buri District, 15.40366°N, 98.6238°E.

***Schistura mahnerti*:** *Mae Klong basin*: Kanchanaburi Province: UF 243489, 4, 27.7–47.1 mm SL, Thong Pha Phum Creek above Nang Khruan Waterfall, Thong Pha Phum District, 14.90512°N, 98.73075°E; UF 192379, 10, 42.4–62.5 mm SL, Song Thai River behind Maharaja field station in Thung Yai Wildlife Sanctuary, Thong Pha Phum District, 15.21071°N, 98.85458°E; UF 245355, 5, 30.0–32.9 mm SL, Ro Khi River W of Ban Sane Phong at Sanaeprong Village, Sangkhla Buri District, 15.221°N, 98.47842°E. Tak Province: UF 246996, 1, 56.7 mm SL, Nam Khiao on Rt. 1090, Umphang District, 16.28778°N, 98.99614°E. *Ataran basin*: Kanchanaburi Province: UF 245437, 10, 41.1–53.7 mm SL, Kasat River at Ban Thi Rai Pa, Sangkhla Buri District, 15.37552°N, 98.58283°E.

Author contributions

L.M.P., S.T., and E.M.K. conceived the study. Data were generated and analyzed by D.K.C., C.V.D., E.M.K., L.M.P., S.T., Z.S.R., and B.R.W. Field work was organized and led by L.M.P., S.T., and W.T. Permits were obtained by S.T., L.M.P., and T.S. Manuscript was prepared by all authors. Funding was generated by L.M.P. S.T., and Z.S.R.

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